

ECOTOXICOLOGICAL EXPERTISE OF WASTE WATER

D. TODOROVA*, L. MECHKUEVA, M. TZANOVA

National Center of Hygiene, Medical Ecology and Nutrition, 15 D. Nestorov Street, 1431 Sofia, Bulgaria

E-mail: detelina_g_todorova@abv.bg

Abstract. The concentrations of heavy metals in the untreated industrial effluent of a copper smelter were analytically estimated. Investigations were carried out in order to determine the dilution rates for untreated industrial waste water with 0, 50 and 100 % mortality of *Daphnia magna* Straus and *Brachydanio rerio* Hamilton-Buchanan, using standard acute toxicity testing methodology. Toxicity test of the treated industrial waste waters was carried out according to the standard procedure. High concentrations of copper, cadmium and arsenic were measured in the untreated effluents. The untreated industrial waste water induced high toxicity for *D. magna* and *B. rerio*. Higher toxicity of water containing copper, cadmium and arsenic with the known single toxicity of each metal were observed. Treated waste water has no toxic effect on *B. rerio* in intermediate-term toxicity tests.

Keywords: waste water treatment, acute toxicity, *Daphnia magna*, *Brachydanio rerio*, heavy metals.

AIMS AND BACKGROUND

Water effluents from metallurgical plants are a major source of pollution of the hydrosphere with heavy metals. The output of non-ferrous metals and their chemical purification produces a large amount of heavy metals that flow in dissolved form in the industrial waste waters.

One way to control heavy metals concentrations in waters is to control their emissions in waste waters.

The temporary functioning of the smelter plant is designed to purify acid waste waters generated from copper and sulphuric acid production until a new treatment plant is constructed. According to the temporary permission the purified waters shall not contain more than 1.5 mg/l arsenic.

The aim of the present paper was to evaluate the effectiveness of the waste water treatment facilities through standard methods for acute toxicity including:

- Determination of levels of dilution of untreated industrial waters at which there is a 0, 50 and 100 % lethal effect on invertebrates and fishes at acute effect in laboratory conditions.
- Determination of the toxic effect of waste waters after treatment by prolonged test on fishes.

* For correspondence.

EXPERIMENTAL

The sample of untreated waste water was collected at the exit of the production before passing through the waste water treatment plant. It has known qualitative and quantitative characteristics: pH, total hardness, high arsenic, copper and cadmium content.

Neutralisation of pH and dilution of the untreated sample with dechlorinated water were carried out before testing.

The sample of treated waste water was collected at the exit of the temporary treatment plant. It has not been pre-treated and diluted.

- Analyses for heavy metals content with atomic absorption spectrometry methods: for copper and cadmium with Flame AAS (Ref. 1); for arsenic – AAS through hydride generation².

- Determination of acute toxicity of untreated waste water with laboratory bred organisms of the type *Daphnia magna* S t r a u s – widely spread zooplankton species in fresh water basins, belonging to class Crustacea, order Cladocera³.

The indicators determined by us were the levels of dilution at which there is 0, 50 and 100 % immobilisation of the animals for 24-hour exposure period.

- Determination of acute toxicity of the sample of untreated waste water with the species *Brachydanio rerio* H a m i l t o n – B u c h a n a n (Teleostei, Cyprinidae)⁴.

The dilution rates at which the mortality rate is 50% of the individuals for each of the monitored periods – 24, 48, 72 and 96 h, as well as dilution rates at which there is 100 and 0% mortality rate at the 24th and 96th hour were determined.

After preparing the solutions and at the end of the test, at some dilution rates adjusting analyses for contents of arsenic, copper and cadmium were performed. No significant changes in their concentrations were established.

The calculated theoretical concentrations of the metals arsenic, copper and cadmium at all dilution rates were compared with the analytically established levels (Table 1).

Table 1. Analytically established and theoretically calculated concentrations of arsenic, copper and cadmium in test solutions*

Metal	Dilution rate (%)														
	10			5			2.5			1.25			0.625		
	tc	ac	% of	tc	ac	% of	tc	ac	% of	tc	ac	% of	tc	ac	% of
	(mg/l)	(mg/l)	tc	(mg/l)	(mg/l)	tc	(mg/l)	(mg/l)	tk	(mg/l)	(mg/l)	tk	(mg/l)	(mg/l)	tc
As	32.7	33.1	101.2	16.35	17.4	106.3	8.18	8.80	107.5	4.08	4.10	100.5	2.04	2.07	101.5
Cu	2.80	-	-	1.40	-	-	0.70	0.67	95.7	0.35	0.33	94.3	0.18	0.15	83.3
Cd	2.36	-	-	1.18	-	-	0.59	0.55	93.2	0.30	0.27	90	0.15	0.13	86.7

Note: *tc – theoretically calculated concentrations; ac – analytically established concentrations.

The mean lethal concentration was calculated – LC 50/s, on the basis of the theoretical total mortality at the end of the test period⁵.

TL50 for 50% of the test animals at different dilution rates was determined.

The χ^2 -test by the Mantel–Haenszel formulae for linear relationship between exposure levels and mortality rate was calculated.

• 14-day test with *Brachydanio rerio* Hamilton – Buchanan (Teleostei, Cyprinidae) of a waste water sample – after being treated at the temporary water treatment plant conducted according to OECD Guideline for testing of chemicals: 204, 1989 (Ref. 6). Lethal and sublethal effects were recorded such as: changes in length and weight, behaviour, swimming, reactions to an external stimulus, reactions at feeding, external appearance of the animals in the tested water sample and were compared to the respective indicators of the control fishes group.

RESULTS AND DISCUSSION

In the preliminary test for assessment of acute toxicity on *Daphnia magna* the inhibition of the mobility of 50% of the daphnia is manifested at dilution rates between 0.156 and 0.078%. The final testing was performed at five dilution rates within this range. The non-effective dilution rate for 24-hour exposure (24 h EC 0) and the level causing 100% immobilisation (24 h EC 100) were obtained directly from the test – at 0.075 and 0.156%. The dilution rate with 50% immobilisation at 24-hour test (24 h EC 50) – 0.11% was obtained at graphic assessment of the results as presented in Fig. 1.

The theoretical concentrations of the metals at dilution rate with 50% immobilisation are 0.37 mg/l arsenic, 0.02 mg/l copper and 0.02 mg/l cadmium.

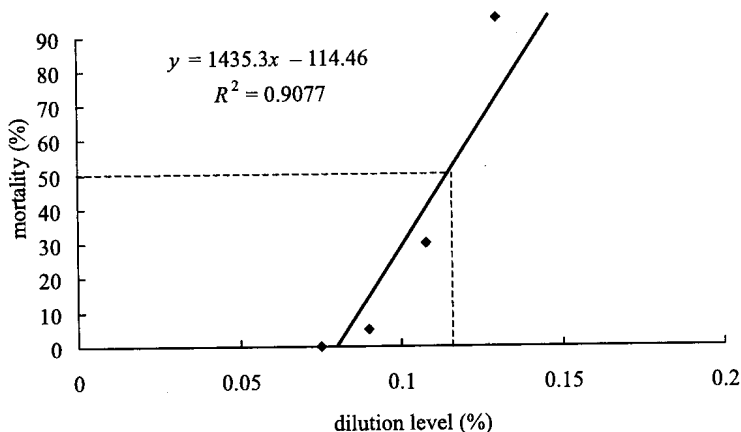


Fig. 1. Relationship between dilution level of waste water and immobilisation of *D. magna*

Irwin et al.⁷ conducting similar tests for acute toxicity on *Daphnia magna* established 24 h EC 50 of arsenic of 1.5 mg/l that is five times greater than the value determined by our studies; the 24 h EC 50 for cadmium was 0.01 mg/l. The literature sources quote 24 h EC 50 values for cadmium compounds within a rather wide range – 0.02 – 0.07 mg/l (Ref. 8); for CdCl₂ it is 1.9 mg/l. The Wasserschadstoff katalog⁹ classifies the metal in Group E containing compounds with toxicity less than 1 mg/l.

Our test results show toxicity of cadmium at the lower limit value – 0.02 mg/l (theoretically calculated concentration of the metal).

Concerning copper toxicity on *Daphnia magna* the Wasserschadstoff katalog⁹ classifies the metal in group E. Chapman¹⁰ established 24 h EC 50 for copper of 0.069 mg/l, at water hardness 207 mg Ca/l.

The acute toxicity test for untreated waste water on *B. rerio* was conducted at eight dilution rates from 0.078 to 10%. The following toxicological parameters were determined: 24 h LC 0 – 0.312%, LC 50 – 1.25% and LC 100 – 2.5%; 48 h LC 50 – 0.312%; 72 h LC 50 – 0.312%; and 96 h LC 0 – 0.156%, LC 50 – 0.312% and LC 100 – 2.5%.

The average lethal concentration – LC 50/s, was calculated on the basis of 50% of the theoretical total mortality during the 96-hour test (Table 2). For this test it was calculated that LC 50/s was 0.36%. The comparison between the obtained LC 50/s and 96 h LC 50 revealed a difference between them of 14%, i.e. less than 30%, thus the result is reliable.

Table 2. Data from testing for acute toxicity on *B. rerio* for determination of LC 50 and average lethal concentration (LC 50/s)

Dilution rate (%)	No of fishes	Results for 96 h			Theoretical total mortality for 96 h			
		dead	alive	% mortality	dead	alive	summary test	% total mortality
10	10	10	0	100	53	0	53	100
5	10	10	0	100	43	0	43	100
2.5	10	10	0	100	33	0	33	100
1.25	10	8	2	80	23	2	25	92.0
0.625	10	10	0	100	15	2	17	88.2
0.312	10	5	5	50	5	7	12	41.7
0.156	10	0	10	0	0	17	17	0
0.078	10	0	10	0	0	27	27	0
0	10	0	10	0	0	37	37	0

TL 50 of 50% of the fishes was determined at the different dilution rates (Fig. 2). For levels from 2.5 to 10% TL 50 is less than 12 h; 24 h are established for 1.25% dilution; 48 h for 0.312%; 36 h is TL 50 for 0.625%; for dilutions 0.156 and 0.078% TL 50 is out of the test period.

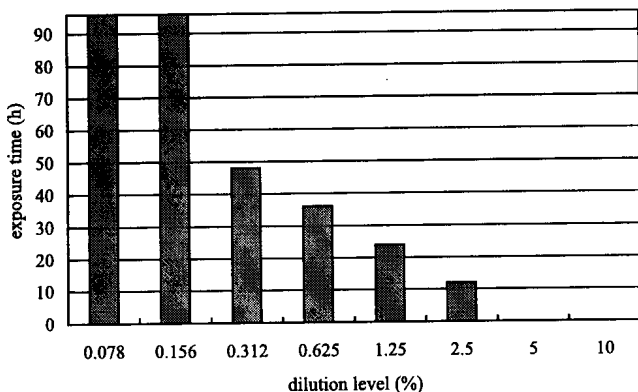


Fig. 2. TL 50 for *B. rerio*

The theoretical concentrations of metals at dilution of 0.312% (96 h LC 50) are arsenic – 1.02 mg/l, copper – 0.087 mg/l, cadmium – 0.07 mg/l.

The *Wasserschadstoff katalog*⁹ places arsenic and cadmium in group D where the elements are toxic for fish in the range 1 – 9 mg/l. According to Irwin et al.⁷, for most fishes arsenic is toxic in a rather wide range – from 1.57 to 41 mg/l. Our test fishes showed greater sensitivity than the quoted evidence.

At testing cadmium toxicity on *B. rerio*⁸ are determined 24 h LC 50 – 7 mg/l and 48 h LC 50 – 4.2 mg/l. In the presented test cadmium concentration at the respective dilution rates is 0.3 and 0.07 mg/l that is substantially lower than those data.

Concerning copper toxicity on *B. rerio* the *Wasserschadstoff katalog*⁹ lists 96 h LC 50 – 0.149 mg/l. Other authors¹¹ outline that copper is toxic for different freshwater fish species in concentrations from 0.03 to 7.3 mg/l.

Statistically significant increase of the mortality rate is established at increasing the exposure levels. The values of χ^2 are 26.09 and $p < 0.01$.

The analytically established concentrations of treated waste water are arsenic – 0.24 mg/l, copper – 0.44 mg/l, cadmium – 0.01 mg/l. The findings at 14-day test on *B. rerio* did not reveal toxic effect associated with treated waste water. The growth rates of the test and control fishes were equivalent. At the end of the test the fish body mass in both groups increased with 0.4 g.

CONCLUSIONS

Untreated waste waters are highly toxic for *Daphnia magna* and *Brachydanio rerio*. The combined exposure to copper, cadmium and arsenic revealed higher toxicity for *D. magna* and *B. rerio* compared to the known individual toxicity of each metal. No toxicity of the treated waste water on *B. rerio* at prolonged exposure was established.

The ecotoxicological tests can successfully be used for assessment of the effectiveness of treatment of waste waters with high content of metals with view to their flowing into natural water bodies used for recreation, fish breeding and irrigation.

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